Magnetic Resonance Spectroscopy as Structural Tool
(electron paramagnetic resonance, nuclear magnetic resonance)

Electron Paramagnetic Resonance and Nuclear Magnetic Resonance are non-invasive techniques which can be applied to living systems in vivo to obtain images, such as distribution of H₂O, O₂, or NO, in tissues.
Magnetic Resonance Spectroscopy

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http://www.cis.rit.edu/htbooks/nmr/
Remember our Goal: From highly resolved 3D & Electronic Structure to Function to Mechanism

EPR = Electron Paramagnetic Resonance
NMR = Nuclear Magnetic Resonance

ABS = UV/Vis
Protein Crystallography
Computational Chemistry

Type 1 Cu
Heme Fe

2Fe-2S
Spectroscopic Techniques - Energy

<table>
<thead>
<tr>
<th>Gamma</th>
<th>X-Ray</th>
<th>UV/Vis</th>
<th>Infrared</th>
<th>Microwave</th>
<th>Radiowave</th>
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<td>2000</td>
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<td>10^{-6} - 10^{-7}</td>
</tr>
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</table>

- Mössbauer
- XAS
- EXAFS
- ABS
- MCD
- CD
- IR
- EPR
- ENDOR
- RAMAN
- NMR

Circular Dichroism
Magnetic Circular Dichroism

EPR + NMR = Electron Nuclear Double Resonance
Spectroscopic Techniques

Energy Source → \( \lambda \) → Sample → B → \( \lambda + \Delta \lambda \)

\( \varepsilon + \delta \lambda \) → EPR

(\(M\))CD

RAMAN
History: The discovery of a new Iron Center

IDENTIFICATION BY ISOTOPIC SUBSTITUTION OF THE EPR SIGNAL AT $g = 1.94$ IN A NON-HEME IRON PROTEIN FROM AZOTOBACTER, Y I SHETHNA, P W WILSON, R E HANSEN, H BEINERT, Proceedings National Academy of Science/USA (1964), 52, 1263-1271

EPR spectra of Fe$^{56}$ and Fe$^{57}$ iron proteins superimposed. The dotted curve represents a computed curve for the Fe$^{57}$ protein, which was obtained from the curve of the Fe$^{57}$ protein, assuming a hyperfine splitting of 22 G and a final enrichment of 65% for Fe$^{57}$.

Helmut Beinert, Madison, Wisconsin, USA
Richard Sands, Ann Arbor, Michigan, USA
Applications of EPR

Which compounds can be studied by EPR? Radicals
Paramagnetic systems with unpaired electrons, \( S \neq 0 \)

In Inorganic Biological Chemistry, Biology, and Medicine

1. Most transition metals: \( \text{Cu}^{II}, \text{Ni}^{I,I,III}, \text{Co}^{II}, \text{Fe}^{III}, \text{Mn}^{II/III/IV}, \text{V}^{IV}, \text{Mo}^{V}, \text{W}^{V} \)
2. Protein side chain radicals (Tyr\(^{•}\), Trp\(^{•}\), Gly\(^{•}\), Cys\(^{•}\))
3. Radical states of cofactors (Semiquinones, Flavins ...)
4. Inorganic radicals (NO\(^{•}\), O\(_{2}\), O\(_{2}^{•-}\), HO\(^{•}\)....)
5. Transient species in light driven processes

...but also

1. Spin Traps can be used to catch short-lived radicals
2. Spin Labels can be attached to proteins, nucleic acids, ... to study their structure and dynamics
EPR - Basic Information

1. **Is the substance paramagnetic?** (Oxidation state of metal ion)
   *Note: Integer Spin Systems might be EPR silent/Technology!*

2. **Which type of paramagnet is present?**
   Fingerprinting! Metal, Organic Radical, Interacting systems

3. **How much paramagnet is present?**
   Quantification!

4. **Geometric and electronic structure of paramagnet**

5. **Information about type and number of ligands**

6. **In interacting systems, information about distances**
Commercial CW-EPR Instrument
sample in 3-4 mm quartz tube; vol. 250 μl; conc. “as much as possible” (0.1 – 0.01 mM)
Important: Cryotechnology/Variable Temperature
Depending on the metal ion liquid N\textsubscript{2} (77K) or He (4.5K)
Basic Properties of Electrons

An Electron has the following properties:

- **Mass** $m_e$
- **Charge** $-e_0$
- **Spin** $\rightarrow$ **Magnetic Dipole Moment** $\mu$

\[
\mu = -g_e \beta \\
2.002319... \text{ Bohr's Magneton}
\]

The Magnetic Dipole in a Magnetic Field:

\[
E = -\mu B = \pm g_e \beta B
\]
An Electron in a Magnetic Field

Energy of an Electron in a Magnetic Field:

\[ E = -\mu B = \pm g_e \beta |B| \cos(\theta) \]

In Quantum Mechanics:

Only Orientations with \( \cos(\theta) = \pm 1/2 \) are possible

Thus, the Electron can have only two states:

\(|+1/2\rangle\) and \(|-1/2\rangle\)

„up“ „down“
The EPR Transition

In order to change the orientation of the electronic magnetic dipole moment in the presence of a magnetic field we need to apply a *FORCE*.

A suitable force is provided by a microwave photon which induces a transition between the $|{-\frac{1}{2}}\rangle$ and $|{+\frac{1}{2}}\rangle$ levels.
Experimental Observation of Resonance

Record the spectrum on a magnetic field scale at fixed frequency $\nu$!

$$\Delta E = g_e \beta \left| B \right| = h \nu$$

$$\left| B \right|_{\text{res}} = \frac{h \nu}{g_e \beta}$$

$h/\beta = 0.7144775$ Gauss/GHz
Presentation of EPR Spectra

The magnetic field is usually measured in **Gauss** (G) units. The SI unit, however, is the **Tesla** (T)!

\[ 1 \text{T} = 10000 \text{ G} \]
\[ 1 \text{ mT} = 10 \text{ G} \]

Typical resonance field

\[ B_{res} \approx 3000 \text{ G} = 0.3 \text{T} \]
Multifrequency EPR

In EPR we usually **FIX** the microwave frequency $\nu$ (because of the cavity) and **VARY** the magnetic field $B$.

The magnetic field scale is inversely proportional to energy!

Thus, for every frequency we need a different Cavity, and we might have to change the magnet:

- **S-Band**: 1-2 GHz
- **C-Band**: 2-4 GHz
- **X-Band**: 9-10 GHz (Standard)
- **Q-Band**: 35 GHz
- **W-Band**: 95 GHz
- **High-Field**: 100-600.. GHz
$g_{\text{effective}} / g_{\text{eff}}$ - bound electrons (atom, molecule)

Resonance does not always occur at the same field: bound electrons carry some ANGULAR ORBITAL MOMENTUM $L$ in addition to the SPIN ANGULAR MOMENTUM $S$.

Additional magnetic moment $\mu_L$

Modification of Resonance Condition:

$$\Delta E = h \nu = -B(\mu_e + \mu_L) = \beta |B| g_{\text{eff}}$$

MOLECULAR Quantity $= g_e + \Delta g$
Anisotropy of \( g \)

The relative orientation of \( B \) and \( \mu = \mu_e + \mu_L \) matters a lot!

Consider three extreme cases:

\[
\begin{align*}
\hbar \nu &= -B_x \mu_x = \beta B_x g_x \\
\hbar \nu &= -B_y \mu_y = \beta B_y g_y \\
\hbar \nu &= -B_z \mu_z = \beta B_z g_z
\end{align*}
\]

Thus, \( g \) becomes anisotropic: the „g-Tensor“
Consequence for the EPR Spectrum

In Bio EPR we usually investigate frozen samples (randomly oriented molecules) and we have to integrate over all possible orientations!

Assume $g_z > g_y > g_x$
„Dialect“ for Powder Patterns

**RHOMBIC**

\[ g_x \neq g_y \neq g_z \]

**AXIAL**

\[ g_x = g_y (= g_\perp) \neq g_z (= g_\parallel) \]

**ISOTROPIC**

\[ g_x = g_y = g_z \]
The Hyperfine Interaction (HFS)

Some Nuclei are *Little Bar Magnets* (→ NMR Spectroscopy)

- The condition is that the Nuclei have a Non-zero nuclear Spin $I$.
  
  \( ^{1,2}\text{H}, ^{14,15}\text{N}, ^{17}\text{O}, ^{19}\text{F}, ^{33}\text{S}, ^{57}\text{Fe}, ^{61}\text{Ni}, ^{63,65}\text{Cu}, ^{77}\text{Se}, ^{95}\text{Mo}, ^{183}\text{W} \ldots \)

- The Magnetic Interaction between the Nuclei and the unpaired Electrons is called Hyperfine Interaction (HFS, Symbol $A$)

- HFS leads to a Splitting of the EPR Lines

**Selection Rule:**

The Nuclear Spin does not change in an EPR Transition

\[ \Delta m_S = 1; \Delta m_I = 0 \]

\( \uparrow \text{Electron Spin} \)

\( \uparrow \text{Nuclear Spin} \)
EPR Spectrum with Hyperfine Structure

Nuclear Spin $I = 1/2$

2$I+1$ Lines

Splitting Depends on the Orientation

$A$ is different for each $g$-direction → „A-Tensor“
Study case 1: Naphthalene Anion Radical in solution; isotropic signal; HFS, $I^H = 1/2$

$4$ equivalent H = Intensity distribution $1:2:3:2:1$

$a_\alpha = 4.9 \text{ G}$

$a_\beta = 1.83 \text{ G}$

Note: Organic radicals usually have g-shifts which are very close to the free electron g-value $g_e = 2.002319$
Study case 2: Iron–Sulfur (FeS) Centers
frozen; 10K; anisotropic

Rubredoxin

[2Fe-2S] Ferredoxin

[2Fe-2S] Rieske center

[3Fe-4S]

[4Fe-4S]

Reduced
$g_{av}=1.96$

Reduced
$g_{av}=1.91$

Reduced
$g_{av}=1.96$

Reduced
$g_{av}=2.01$

Reduced
$g_{av}=1.96$

Reduced
$g_{av}=2.06$

Oxidized
$g_{av}=2.01$

Oxidized
$g_{av}=2.02$

Oxidized
$g_{av}=1.96$

Oxidized
$g_{av}=2.06$

$g_x=1.79$

$g_y=1.92$

$g_z=2.02$

$g_x=1.88$

$g_y=2.00$

$g_z=2.04$

$g_x=1.79$

$g_y=1.95$

$g_z=2.05$

$g_x=1.89$

$g_y=2.00$

$g_z=2.02$

$g_x=1.95$

$g_y=1.92$

$g_z=2.12$

$g_x=2.12$

$g_y=2.04$

$g_z=2.04$

$g_x=1.79$

$g_y=1.95$

$g_z=2.05$

$g_x=1.89$

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$g_y=1.92$

$g_z=2.12$

$g_x=2.12$

$g_y=2.04$

$g_z=2.04$

$g_x=1.79$

$g_y=1.95$

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$g_y=1.92$

$g_z=2.12$

$g_x=2.12$

$g_y=2.04$

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$g_y=2.04$

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$g_x=1.79$

$g_y=1.95$

$g_z=2.05$

$g_x=1.89$

$g_y=2.00$

$g_z=2.02$
Study case 3: Type 1 Blue Cu vs Cu(II) complex frozen; 77K; axial spectrum; $I^Cu = 3/2$

<table>
<thead>
<tr>
<th>Blue Copper Sites in Poplar plastocyanin</th>
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<tbody>
<tr>
<td><strong>X-ray Structure</strong></td>
</tr>
<tr>
<td><strong>Absorption</strong></td>
</tr>
<tr>
<td><strong>EPR</strong></td>
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Hans Freeman, 1978

Magnetic Resonance Spectroscopy as Structural Tool

Nuclear Magnetic Resonance

With lecture notes from
Dr. Christina Drees
AG Möller, University of Konstanz
Applications

– Substance identification
– Purity
– Fingerprint
– Analysis of conformation
– Determination of 3D structure (proteins, RNA, DNA)
Theory

• Nuclei with impair nuclear charges and impair mass numbers possess a spin, like electrons
• Commonly studied nuclei: \(^1\)H, \(^{13}\)C, \(^{15}\)N, \(^{19}\)F, \(^{31}\)P

http://arrhenius.rider.edu/nmr/nmr_tutor/periodic_table/nmr_pt_frameset.html
Theory

- Magnetic moments have a direction

Without magnetic field:

With magnetic field:

- Alignment parallel (α) or antiparallel (β) to external magnetic field
• Measurement of $\Delta E$ (difference in energy)

• Population difference only 1/100.000
Acquisition of NMR spectra
Sample preparation

- 1 mg Sucrose solved in 600 μl water

(H₂O / D₂O 9:1)
Configuration of a NMR magnet

Superconductive coil of wire in liquid helium (4 K)

Isolated by vacuum

Isolated by liquid nitrogen (77 K)

Again isolated by vacuum
Theory

• Absorption of electromagnetic radiation $E = h \times \nu$ to induce a transition between the $\alpha$ and $\beta$ energy levels

If the frequency of the radio waves ($E = h \times \nu$) matches exactly $\Delta E$, -> absorption of the energy; Transition from $\alpha$ to $\beta$; Measurement of the absorbed frequency with a detector
$^1\text{H-NMR}$ Spectrum of Sucrose
Theory

Electron magnetic field counteracts the external magnetic field
This magnetic field shields the nucleus
-> Shielding by electrons
Nucleus which is shielded with a higher electron density needs lower energy (lower frequency) for transition from $\alpha$ to $\beta$

-> Information about the chemical environment
$^1$H-NMR Spectrum of Sucrose

- Chemical shifts
- Resonance intensities (Integration)
- Spin-spin coupling
Chemical shift

- Reference for chemical shift $\delta$: TMS (Tetramethylsilan)
- Definition $\delta$ (TMS) = 0
- Frequencies relative to the resonance frequency of TMS in parts per million (ppm)
  - independence of the magnetic field strength
Chemical shift

- $\delta$ depends on electron density around the nucleus

High frequency
low electron density
$\rightarrow$ shielded

Low frequency
high electron density
$\rightarrow$ shielded
Chemical shift

CH$_3$–CH$_2$–OH
Ethanol

ppm
Quantitation - Resonance Integration

- Integral of a resonance is proportional to number of protons
Spin-spin coupling

• Splitting of signals = coupling

- Information, how many hydrogen atoms are in the neighborhood of the observed nucleus
- Coupling over at most 3 – 4 chemical bonds
- Protons that are equivalent show no coupling among themselves
Spin-spin coupling

- One signal splits in \((n + 1)\) lines, when coupling to \(n\) equivalent protons
- Line intensities according to Pascal’s triangle

<table>
<thead>
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</table>

**Fig. 4.22** Pascal’s triangle. Coupling to \(n\) equivalent spin-\(\frac{1}{2}\) nuclei produces \(n + 1\) lines, the relative intensities of which are given by the triangle.
Spin-spin coupling

- Distance between two lines in a multiplet: coupling constant $J$ in Hz

- Multiplets of coupling nuclei have the same coupling constant $J$
Fru-H3
Fru-H4
Glc-H3
Glc-H4
Glc-H2
Fru-H1_{ab}
Fru-H6_{ab}
Glc-H5,H6_{ab}
$^{13}$C-NMR

- Natural abundance of 1.1 % and low sensitivity
  → Acquisition takes more time

- No couplings observed
  - $^{13}$C-$^{13}$C couplings because of low natural abundance
  - $^1$H-$^{13}$C couplings because of decoupling during acquisition

- No integration because of decoupling

→ Determination of the number of different carbon atoms in a molecule
$^{13}$C Chemical shifts
$^{13}$C-NMR Spectrum of Sucrose
NMR STUDIES OF STRUCTURE AND FUNCTION OF BIOLOGICAL MACROMOLECULES

Nobel Lecture, December 8, 2002
KURT WÜTHRICH

Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland, and The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA.

Figure 1. NMR structure of the Antennapedia homeodomain. A bundle of 20 superimposed conformers represents the polypeptide backbone. For the polypeptide segment 7–59 the tight fit of the bundle indicates that the structure is defined with high precision, whereas the two chain ends are disordered.

- Protein expression
  - Optimize expression yield
  - Optimize for high concentration, solubility, stability
  - Express $^{15}$N- and $^{15}$N-/$^{13}$C-labelled protein
Protein structure

$^1$H NMR

→ Overlap

→ 2D / 3D experiments
2D Spectrum
Protein structure

• Sequential Assignment: identify amino acid spin systems and their sequence position
Protein structure

- Side chain assignment: determine all relevant chemical shifts of $^1H$, $^{13}C$ and $^{15}N$
Structure Calculation

Interproton distances (NOEs) and dihedral angles; NOE = Nuclear Overhauser Effect, highly useful for characterizing and refining organic chemical structures; in this application, the NOE differs from the application of spin-spin coupling in that the NOE occurs through space, not through chemical bonds. Thus, atoms that are in close proximity to each other can give a NOE, whereas spin coupling is observed only when the atoms are connected by 2–3 chemical bonds. The inter-atomic distances derived from the observed NOE can often help to confirm a precise molecular conformation, i.e. the three-dimensional structure of a molecule.
From analysis of NOEs to 3D structure of Zn protein